Chapter-III

Evaluation of potential EPNs against different foraging behavior test insects (*Eupterote mollifera*, *Helicoverpa armigera* and *Eutectona machaeralis*)

1. INTRODUCTION

Insect pests are a major cause of crop loss, the problem in deal with pest management is inadequate knowledge about the factors influencing pest population dynamics. To understand pest dynamics, pest surveillance and related agricultural operations regarding crops, farming practices and other weather parameters are essential and predominantly details of pest incidence, climatic, soil, agricultural practices. Many selective pesticides are directly toxic to pest species only, their use can cause disruption to non target beneficial organisms by reducing their insect hosts and ravenous the beneficial species or prompting them to migrate out of the field. It is indirect reduction of beneficial may allow the remaining pest population to increase to higher levels if their reproductive potential go beyond that of the remaining beneficial. Many caterpillar pests infest vegetable crops. An organic farming system offers many opportunities for enhancing biological control factors. While most organic farms apply little or no harsh pesticides, it is possible to build up large numbers of beneficial parasites and predators that facilitate control pests in many crops. Multiple releases of biological control agents may be required in order to bring pest populations to very low levels.

Biological control is a valuable method for pest management; natural enemies can actively seek out pests in unattainable site, and also it can be applied in a manner similar to chemical pesticides. EPNs are lethal endoparasites of insects (Gaugler and
Kaya, 1990; Gaugler, 2002) that kill the host within 24–48 h. EPNs have been using as biological control agents in the soil environment (Kaya and Gaugler, 1993). They have many significant characteristics that make them potential biological control agents mainly low toxicity to vertebrates (Bathon, 1996; Boemare et al., 1996; Kaya and Gaugler, 1993), they are free from registration in the United States by the EPA (Kaya and Gaugler, 1993), several species are commercially available (Grewal, 2002), they can be applied with conventional pesticide equipment (Hayes et al., 1999), they can tolerate from few kind of pesticide (Koppenhofer et al., 2000; Nishimatsu and Jackson, 1998), wide host range (Capinera and Epsky, 1992; Gaugler et al., 1997).

*Helicoverpa armigera* is one of the key pests causing rigorous yield losses, infesting several crops such as cotton, maize, chickpea, pigeonpea, sunflower, soyabean and groundnuts (Fitt, 1989). Ecological and physiological features like high fecundity, multi-voltinism, ability to migrate long distances and diapause during unfavorable conditions contribute for it's severity in different situations. In India, the cotton crop provides ecological niche to the bollworm, *H. armigera*. Among the various options for their management, the use of insecticides is the currently dominant tool. The random use of insecticides has resulted in resistance development of *H. armigera* to almost all groups of insecticides. In India, resistance was first reported in cotton growing belt of Andhra Pradesh in 1987. Low to moderate levels of resistance have been reported in Maharashtra, moderate to high in Tamil Nadu. *S. masoodi* against gram pod borer, *H. armigera* infesting chickpea under field conditions was studied (Ahmad, 2003). Three species of EPNs *H.indica*, *H. bacteriophora* and *S. glaseri* was considered against III, IV and V instars *H. armigera*. *S. glaseri* was the most effective strain in causing mortality.
Eutectona machaeralis is known as leaf skeletonizer, the most widespread and serious pest in teak plant. Occurrence almost every year in the early flushing period and trees suffer a total defoliation, occasionally there is partial defoliation later in the growth season (Nair, 1988). Bio-insecticides / fungicides were used against E. machaeralis. They can be controlled by using quinalphos as spraying, synthetic pyrethroids, Bacillus sp. so far studied in the laboratory condition. Still more investigation is needed on the hibernation behaviour as well as on its occasional widespread population outbreak and control strategy using biocontrol agents. Studies on EPNs species against E. machaeralis are modest.

Drumstick tree, also known as horseradish tree Moringa. Drumstick tree has numerous traditional medicinal uses (Ramachandran et al., 1980; Parrotta, 2001). M. oleifera is susceptible to several insect pests. These include the bark-eating caterpillar, Indarbela quadrinotata WLK.; the hairy caterpillar, Eupterote mollifera WLK. The susceptibility of different larval instars of E. mollifera to 3 commercial B. thuringiensis (Bt), formulations (Spicthurin, Spic Bio and Delfin) were investigated. The infectivity of the S. glaseri against the moringa hairy caterpillar, E. mollifera was observed.

Subramaniam, (2000) was studied invivo production of selected insects for IJs production in under laboratory C. cephalonica, G.mellonella, H. armigera, Spodoptera litura, E.mollifera, Dichocrocis punctiferalis, Chilo sacchariphagus indicus, Cylas formicarius and Periplaneta americana, recorded the highest IJs production of H. indica and S. glaseri.
The exposure of EPNs on foliage to extreme temperature (Grewal et al., 1994), ultraviolet light (Gaugler et al., 1992), and rapid fluctuation in moisture that causes desiccation (Baur et al., 1995) reduces their potential as biocontrol agents against foliage-feeding insects. EPNs can be applied, in combination with other biological and chemical pesticides, fertilizers and soil amendments and in the form of adjuvants or antidesiccants (Glazer and Navon, 1990; Baur et al., 1997). Foliar applications of nematodes have been successfully used to control the quarantine leaf eating caterpillars on various crops and have the potential for controlling various other insect pests. In the present study, the selected potential strains were evaluated for host preference assay against different foraging behavior insects such as H. armigera, E. machaeralis and E. mollifera.

2. MATERIALS AND METHODS

2.1. Nematode culture

The four potential strains (Steinernema sp. 3 Nos and Heterorhabditis sp. 1 No.) were cultured in the fifth instar larvae of G. mellonella following the Dutky et al., (1964) technique. The infective juveniles were collected using White trap method (White, 1927) and were stored at 15ºC in BOD incubator for further analysis.

2.2. Rearing of H. armigera (Lepidoptera: Noctuidae)

The H. armigera larvae were used in this study as test insect. Initially the larvae were collected from the cotton fields, later the larvae were maintained and reared in the semi-synthetic diet (Armes et al., 1992) separately in the laboratory conditions. Larvae were provided fresh diet until pupation. Freshly formed pupae were transfer to insect rearing cage (30cm X 30cm) having moist sponge and blotting paper at the bottom,
covered with muslin. Temperature of laboratory was maintained up to 25±2°C. After emerging of the adults moths were encouraged for mating. After mating the females were allowed to egg laying in the cotton leaves with twigs dipped in the water containers. Finally the eggs were removed daily and kept in glass jars for hatching. The neonate larvae were reared individually and used for the experimental purpose. Third and fifth instar larvae were selected for bioassay studies.

2.3. Rearing of *E. machaeralis* (Lepidoptera: Hyblaeidae)

The *E. machaeralis* larvae were used as test insect in this study. Primarily, the larvae were collected from the teak plants in the Bharathidasan University Campus, Tiruchirappalli, Tamil Nadu. The collected *E. machaeralis* larvae were transferred to the laboratory and reared in the teak leaves for experimental study. The matured larvae were allowed to pupation and adult emergence for egg laying. The eggs were monitored until hatching, further the first and second instar larvae were fed with young teak leaves, under protection of strand and silk. The third and fifth larvae were used for bioassay studies.

2.4. Rearing of *Eupterote mollifera*

*Eupterote mollifera* larvae were used in this as a test insect. Initially the larvae were collected from the *Morniga oleifera* fields in and around Tiruchirappalli District, Tamil Nadu, India. The collected *E. mollifera* larvae were transferred to the laboratory and reared in *M. oleifera* leaves for experimental purpose. The matured larvae were allowed to pupation and adult emergence for egg laying. The eggs were monitored until hatching, further the first and second instar larvae were fed with young Moringa leaves. The emerging moths were fed with 10% sucrose solution fortified with a few drops of
vitamin mixture (multidec drops) to enhance oviposition. The eggs were collected and hatched out. The larvae were reared in the tray and fed with leaves. The third and fourth instars larvae were collected freshly for bioassay study.

2.5. **Bioassay (Filter paper Method)**

In the laboratory conditions the isolated potential strains were evaluated for pathogenicity study in different foraging behaviour insects, such as *H. armigera*, *E. machaeralis* and *E. mollifera*. The pathogenicity assay was conducted in the filter paper method (Kung *et al*., 1990). Totally 10 mature (third-and fifth instar) larvae were placed separately on filter paper disk immersed in the 0.1% formaldehyde in 6 cm-diameter plastic petri dishes. In addition the filter paper disks were wetted with 2 ml of suspension (200 IJs). All the experiments were placed in plastic tray and incubated at room temperature 25±2°C. After 24 h larval mortality was recorded. All dead larvae were collected and transferred to individual white trap (White, 1927) for the IJs emergence (offspring production). Moreover, untreated control was maintained in 1ml of distilled water without isolates (IJs). There were three replicates for each experiment conducted.

2.6. **Reproduction potential**

After bioassay study all the cadavers (Bdu-173, Bdu-401, Bdu-85 and Bdu-146) were collected individually and kept in the white trap (White, 1927) for the IJs emergence (offspring production). The dishes were kept in a moist container at 25±2°C for up to 5-12 days. The emerged IJs were collected individually and rinsed in a glass beaker to remove debris and the IJs populations were counted using stereo zoom microscope.
2.7. **Statistical analysis**

The values are expressed as mean ± SD for three replicate in each assay. The differences between isolates were assessed by one-way ANOVA using SPSS software package for Windows (Version 11.5; SPSS Inc., Chicago, IL, USA). Post-hoc testing was performed for intergroup comparison using the least significance difference test. Values of $P<0.001$, 0.01 and 0.05 have been denoted by distinct symbol in the tables.

3. **RESULTS**

3.1. **Pathogenicity of *Helicoverpa armigera***

The potential strains *Steinernema* sp. (Bdu-173, Bdu-401, Bdu-85) and *Heterorhabditis* sp. (Bdu-146) were assessed against three different foraging insects. In present study *H. armigera* was used as test insect to identify the host specific and their potential development. Pathogenicity was conducted against III and V instar larvae of *H. armigera* and it was statistically significant. In our observations results suggests that out of four isolates Bdu-401 showed less pathogenicity against III and V instar larvae but Bdu – 85 showed 100% mortality (Table 16, Plate 4).

3.2. **Pathogenicity of *E. machaeralis***

The potential strains Bdu-173, Bdu-401, Bdu-85 and Bdu-146 were assessed against *E. machaeralis* larvae. In present study pathogenicity was conducted against III and V instar larvae of *E. machaeralis* and it was statistically significant. In our observations results suggest that among the four isolates, Bdu-401 showed less pathogenicity against III and V instar larvae but Bdu-85 showed cent percent mortality (Table 17, Plate 4).
3.3. **Pathogenicity of *E. mollifera***

The selected four strains Bdu-173, Bdu-401, Bdu-85 and Bdu-146 were assessed pathogenicity against III and V instar larvae *E. mollifera* larvae. In present study larval mortality was statistically significant in all the nematode treatment. In 24 h treatment Bdu-401 showed 42.49±1.18% Bdu-173 (51.3±1.19%) Bdu-85 (72.6±1.47%) and Bdu-146 (62.76±0.80%) pathogenicity against III instar larvae and Bdu-401 (51.31±1.19%) Bdu-173 (59.90±1.57%) Bdu-85 (79.40±1.25%) and Bdu-146 (68.33±1.06%) showed against V instar larvae. In 48 h treatment Bdu-85 showed 100% pathogenicity against III and V instar larvae, but Bdu-401 strain recorded less pathogenicity (Table 18, plate 4). Interestingly In our observation all the potential strains proved its potentials against V instar larvae than the III instar larvae. Moreover when compared to three insect host the potential nematode strains easily kills the *E. mollifera*.

3.4. **Reproduction potential**

In this study the offspring production was counted against three different insect pests (*E. mollifera*, *H. armigera*, *E. machaeralis*). The Bdu-146 showed higher progeny production in the entire three insect host (*E. mollifera*, III instar-80,100 and V instar -85,400, *H. armigera*, III instar-1,04,125 and V instar -1,18,426, *E. machaeralis*, III instar - 61,316 and V instar – 65,412,) but Bdu-401 showed less progeny production (*E. mollifera*, III instar-17,200 and V instar -21,250, *H. armigera*, III instar-28,423 and V instar -31,720, *E. machaeralis*, III instar – 8,120 and V instar – 9,450). The result suggest that among the three different insects *H. armigera* recorded high progeny production (Bdu-401 III instar-28,423 and V instar – 31,721, Bdu-173 III instar- 80,100 and V instar – 94,450, Bdu-85 III instar-54,645 and V instar – 60,100,
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<th>V instar</th>
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<td>24h</td>
<td>48h</td>
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<tr>
<td>Bdu-401</td>
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<td>51.46±1.15</td>
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<td>Bdu-173</td>
<td>41.52±0.99</td>
<td>60.00±1.69</td>
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<td>Bdu-85</td>
<td>62.00±1.86</td>
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<td>Bdu-146</td>
<td>57.23±1.05</td>
<td>81.23±0.56</td>
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Values expressed as mean ± SD of three replicate in each test
Statistical analyses (*, represent P< 0.001)

a= 24 h of III Instar vs 24 h of V Instar
b= 48 h of III Instar vs 48 h of V Instar
c= 72 h of III Instar vs 72 h of V Instar
Tabe 17: Assessment of parasitic activity of recovered potential isolates against III and V instar larvae of *E. mahaeralis* at 25°C±2 °C 2°C (20 IJs / larva)

Values expressed as mean ± SD of three replicate in each test
Statistical analyses ( *, and # represents P<0.001 and p<0.01 respectively)

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<td>24h</td>
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<td>Bdu-401</td>
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<td>62.31±1.07</td>
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<td>45.88±1.40 b*</td>
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<td>Bdu-85</td>
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<td>100 ±0.00</td>
<td>45.42±1.29 a#</td>
<td>68.36±1.10 b*</td>
<td>97.47±1.14 c*</td>
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<td>Bdu-146</td>
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<td>100 ±0.00</td>
<td>43.70±0.51 a*</td>
<td>62.40±1.04 b*</td>
<td>87.15±1.64 c*</td>
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a= 24 h of III Instar vs 24 h of V Instar
b= 48 h of III Instar vs 48 h of V Instar
c= 72 h of III Instar vs 72 h of V Instar
d= 96 h of III Instar vs 96 h of V Instar
Table 18: Assessment of parasitic activity of recovered potential isolates against III and V instar larvae of *E. mollifera* at 25°C ± 2°C (20 IJs / larva)

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<th>Isolates code</th>
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<tr>
<td>Bdu-401</td>
<td>42.49±1.18</td>
<td>62.18±0.43</td>
<td>100±0.00</td>
<td>100±0.00</td>
<td>51.31±1.19 a*</td>
<td>68.98±1.56 b*</td>
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<td>100±0.00</td>
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<tr>
<td>Bdu-173</td>
<td>51.31±1.19</td>
<td>65.55±3.85</td>
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<td>100±0.00</td>
<td>59.90±1.57 a*</td>
<td>75.41±0.81 b*</td>
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<tr>
<td>Bdu-85</td>
<td>72.6±1.47</td>
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<tr>
<td>Bdu-146</td>
<td>62.7±0.80</td>
<td>91.87±1.65</td>
<td>100±0.00</td>
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<td>68.33±1.06 a*</td>
<td>97.58±0.62 b*</td>
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Values expressed as mean ± SD of three replicate in each test
Statistical analyses (*, represent \( P< 0.001 \))

a= 24 h of III Instar vs 24 h of V Instar
b= 48 h of III Instar vs 48 h of V Instar
Plate - 4
Pathogenicity of recovered potential isolates against three different foraging behaviour insect pests

A. Larvae of Euphytophthora mollifera
C. Larvae of Helicoverpa armigera
E. Larvae of Euteectona machaeralis

B. EPNs infected larva (E. mollifera)
D. EPNs infected larva (H. armigera)
F. EPNs infected larva (E. mecharealis)
Figure 7. Average No. of offspring production of Potential isolates in in *E. mollifera*

Figure 8. Average No. of offspring production of Potential isolates in *E. machaeralis*

Figure 9. Average No. of offspring production of Potential isolates in *H. armigera*
Bdu-146 III instar-1,04,125 and V instar – 1,18,426) and *E. machaeralis* recorded less progeny production in III and V instar larvae. Therefore, significant difference was observed in the rate of infectivity and production and this might be indistinct in the nematode life cycle (Fig. 7 to 9).

4. DISCUSSION

Biocontrol agents used against insect pests, it is critical to match with suitable host to control. Since some controlling agents have a limited host range and host specific. Biological control agents have encouraged most of the researchers particularly on EPNs Steinernematidae and Heterorhabditidae are parasites of insects that kill their hosts with the aid of symbionts. These EPNs will promote the sustained use of agriculture and develop a better understanding the persistence distribution. Due to the outstanding successes made with other biological control agents, our research is poised for developing novel ideas to execute the use of EPNs and their behavior and ecology have been studied to develop better biological control agents. Ecological factors such as relative ability to withstand desiccation or temperature tolerance are also important in choosing the best-adapted nematode for a particular pest. Poor host suitability has been the most common cause of failure in EPNs application. In addition, high virulence under laboratory conditions has often been inappropriately extrapolated to field conditions (Georgis and Gaugler, 1991).

Studies have also shown that EPNs applications do not have a significant impact on non target insects. Soil nematodes can be used as biological control agents to suppress a variety of economically important insect pests (Grewal *et al.*, 2005; Kaya and Gaugler, 1993; Klein, 1990; Shapiro-Ilan *et al.*, 2002). Effective and efficient
delivery of EPNs can only be achieved with careful consideration of available application technology and understanding of the attributes and limitations. Since, insects are able to defend themselves against foreign organisms by their own immune response (reviewed in Gillespie et al., 1997). Proper match of the EPNs to the insect pests, entail virulence, host finding, and ecological factors are essential before application to the field level. If a nematode does not possess a high level of virulence toward the target pest, there is little hope of success. In rare cases, persistence may compensate for moderate virulence (Shields et al., 1999). Matching the appropriate nematode host-seeking strategy with the pest is also essential. Nematodes that have an ambush strategy are most suitable for controlling mobile insects near the soil surface (*S. carpocapsae*), whereas nematodes with a cruiser strategy (*H. bacteriophora*) are most effective for less mobile insects below the soil surface (Lewis et al., 1992). In the EPNs application *S. scapterisci* is effective against mole crickets, but not against other insects (Nguyen and Smart, 1991).

Hominick and Reid, (1990) suggested that the soil nematodes with the greatest efficacy against target insect pests would have the highest invasion efficiency as a direct measure of nematode infectivity or insect pathogenicity. If the EPNs were less efficient, the efficiency against a given host partly depends on the host-finding ability and the penetration capability of the IJs (Ishibashi and Kondo, 1990; Peters and Ehlers, 1994). Shankaranarayanan et al. (2006) reported that the morphological and physiological defense strategies of insects might also affect the nematode ability to infect the host and multiply. In the present study the nematode-host contact had been maximized on the filter paper. In another explanation could be a restricted entry of the IJs through natural openings and the cuticle of the adult beetles. In the present investigation on the pathogenicity assay on *Steinernema* sp. (Bdu-173, Bdu-401, Bdu-
85) and *Heterorhabditis* sp (Bdu -146) against various instar larvae resulted in increased percent mortality with an increased age of larva and duration of exposure. In the series of laboratory experiments, mortality of pests varied when exposed to different EPNs species, the greatest mortality was caused by *Steinernema* sp. Bdu-85 against III and V instar larvae of three different foraging behaviour insects. In our observations, even though the Bdu-401 strain has come out with successful agent among 25 isolates but it was ranked behind the four potential strains tested for host suitability against different foraging insects.

In the pathogenicity study, among the nematodes treatment, Bdu-85 has highest percentage mortality was recorded against *E. mollifera* at 48 h, *H. armigera* at 72 h and, *in E. machaeralis* at 96 h respectively. In our observations the later stage larva infectivity percentage was more since, the over growth, natural opening (anus, spiracle, cuticle and mouth) and feeding behaviour of the larva. Among the suitable host finding analyze against three different insects EPNs pathogenicity showed statistically significant. In the overall experiment the offspring production of isolated strains were compared, the *Heterorhabditis* sp. (Bdu-146) produced more number of IJs against (*E. mollifera*, III instar-80,100 and V instar -85,400, *H. armigera*, III instar-1,04,125 and V instar -1,18,426, *E. machaeralis*, III instar - 61,316 and V instar – 65,412) all the larval stages in different foraging behavior insects since the morphology measurements was smaller when compared to the other strains. But in *Steinernema* sp. (Bdu-401) showed very less no. of progeny production. Moreover, the overall treatment resulted *H. armigera* recorded more no. of progeny production (Bdu-401 III instar-28,423 and V instar – 31,721, Bdu-173 III instar- 80,100 and V instar – 94,450, Bdu-85 III instar-54,645 and V instar – 60,100, Bdu-146 III instar-1,04,125 and V instar – 1,18,426) and in *E. machaeralis* recorded less number of progeny production.